Practical use of hind leg banding patterns for identifying members of the *Anopheles gambiae* group of mosquitoes

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ABSTRACT. Quantitative evidence is provided in support of using the pale band at the apex of hind tarsus 3 and base of hind tarsus 4 to separate *Anopheles gambiae* and *Anopheles arabiensis* from *Anopheles merus* and *Anopheles quadriannulatus* in southern Africa.

INTRODUCTION

A preliminary report by Coetzee et al. (1982) showed the potential use of hind leg banding characters for separating certain species of the *Anopheles gambiae* group in southern Africa. It showed that the pale bands on hind tarsi 3 and 4 in the major malaria vectors *gambiae* and *arabiensis* were generally narrower than those of the sibling species *merus* and *quadriannulatus* where the pale bands tended to overlap the joints of the adjacent segments. *Anopheles quadriannulatus* is not considered to be of any importance medically and *merus* of only minor importance (see White 1974).

This paper is an extension of the preliminary report by Coetzee et al. (1982) and presents quantitative data from new localities.

MATERIAL AND METHODS

Most of the material used by Coetzee et al. (1982) was used here in addition to more recent samples collected. The localities sampled are: *gambiae* Mahongo, Namibia (18°05'S, 21°45'E), Grand Comoros (11°40'S, 43°16'E), Yaka Yaka near Brazzaville, Congo (04°22'S, 15°09'E); *arabiensis*, Pelindaba, Zululand (27°05'S, 32°33'E), Komatipoort, Transvaal (25°26'S, 31°58'E), Jaffray, Transvaal (23°50'S, 30°20'E) all in South Africa, Kanyemba, Zimbabwe (15°40'S, 30°20'E), Nsoro, Swaziland (26°40'S, 31°56'E), Mahongo, Namibia; *merus*, Soutini, Transvaal (23°26'S, 30°54'E), Makanis Drift, Zululand (27°02'S, 32°19'E), Opansi, Zululand (27°34'S, 32°18'E), Shemula, Zululand (27°05'S, 32°17'E), Kosi Bay, Zululand (26°55'S, 32°55'E), all in South Africa; *quadriannulatus*,

Constantia (23°35'S, 30°35'E), Hoogmoed (23°20'S, 30°10'E), Komatipoort, all in Transvaal, South Africa, Makanis Drift and Shemula, Zululand, South Africa, Kanyemba, Zimbabwe.

All new collections were identified chromosomally (Coluzzi 1968, Green 1972, Green & Hunt 1980) or electrophoretically (Mahon et al. 1976, Miles 1978, 1979).

The morphological material used in this study is housed in the collections of the South African Institute for Medical Research (SAIMR). Photographs of chromosomes and electromorphs of the Grand Comoros and Congo material are also housed in the SAIMR reference collection.

Measurements of the pale band at the junction of hind tarsomeres 3 and 4 were taken using an eyepiece micrometer on a stereo microscope.

RESULTS AND DISCUSSION

A total of 514 females were examined; 164 *gambiae*, 86 *arabiensis*, 115 *quadriannulatus* and 149 *merus;* in all, 805 hind legs were measured. The graph in Fig. 1 shows the overlap between the two groups *gambiae/arabiensis* and *merus/quadriannulatus*. 228 females had pale bands on both legs narrower than 0.099mm = *gambiae/arabiensis;* 255 females had pale bands on both legs wider than 0.1mm = *merus/quadriannulatus;* 11 females had one hind leg measurement in each of the above catagories and could not be identified; 20 females had both leg measurements in the wrong category and would have been misidentified. 94% of the total sample were grouped correctly. Of the four species examined, *arabiensis* showed the most variability and only 83.7% of the sample could be grouped correctly.

Material in museum collections around the world have been examined but have not been included in this study. In many cases the specimens were collected and deposited prior to the elucidation of the *gambiae* complex (Paterson, 1964) and are therefore unidentified. In most other cases the method of identification is not indicated.

The types of *gambiae* Giles and *quadriannulatus* Theobald were examined. Both specimens are damaged and only have one hind leg each. The hind leg band of *gambiae* measures 0.03mm and that of *quadriannulatus* 0.04mm which places both of them in the *gambiae/arabiensis* category. The fact that the measurement of *quadriannulatus* does not fall into the *merus/quadriannulatus* group may indicate that the type specimen is not of the species defined genetically and now called *quadriannulatus*.

The *Anopheles gambiae* complex is a particularly difficult group of mosquitoes to study because of the lack of morphological characters by which they can be separated. The techniques used to identify the species (see Materials and methods) are not simple in the classical morphological sense, but are essential for the study of this important group of mosquitoes. Unfortunately, the studies

conducted on the *gambiae* group using proper identification methods have not often included the preservation of morphological specimens. The paucity of identified wild material in museum collections is to be regretted.

The separation of *merus* from *gambiae* and *arabiensis* using morphological criteria has been demonstrated by previous workers. For example, Coluzzi (1964) showed that the palpal ratio and number of coeloconic sensilla on the antennae were significantly different for *merus*. Bushrod (1981) confirmed that the combination of these two characters gave almost 100% identification of *merus*.

Only three papers report on the adult morphology of *quadriannulatus*. Paterson et al. (1963) reported a low frequency of 4-banded palps; Ismail & Hammoud (1968) gave the number of coeloconic sensilla on the female antennae; Green (1971) examined the spermatheca size. All three reported that *quadriannulatus* could not be separated from *gambiae* or *arabiensis*. As *quadriannulatus* has not been incriminated as a vector of malaria, it is important for practical malaria control to be able to separate it from the two main vectors. If this can be done using morphological characters it becomes much easier for the field entomologist to identify populations and to decide on control programmes.

It is possible that the measurements reported on in this paper apply only to the localities sampled and not to other areas in Africa. For this reason it is important to correlate measurements with chromosomally identified material before this technique is applied in an unstudied area.

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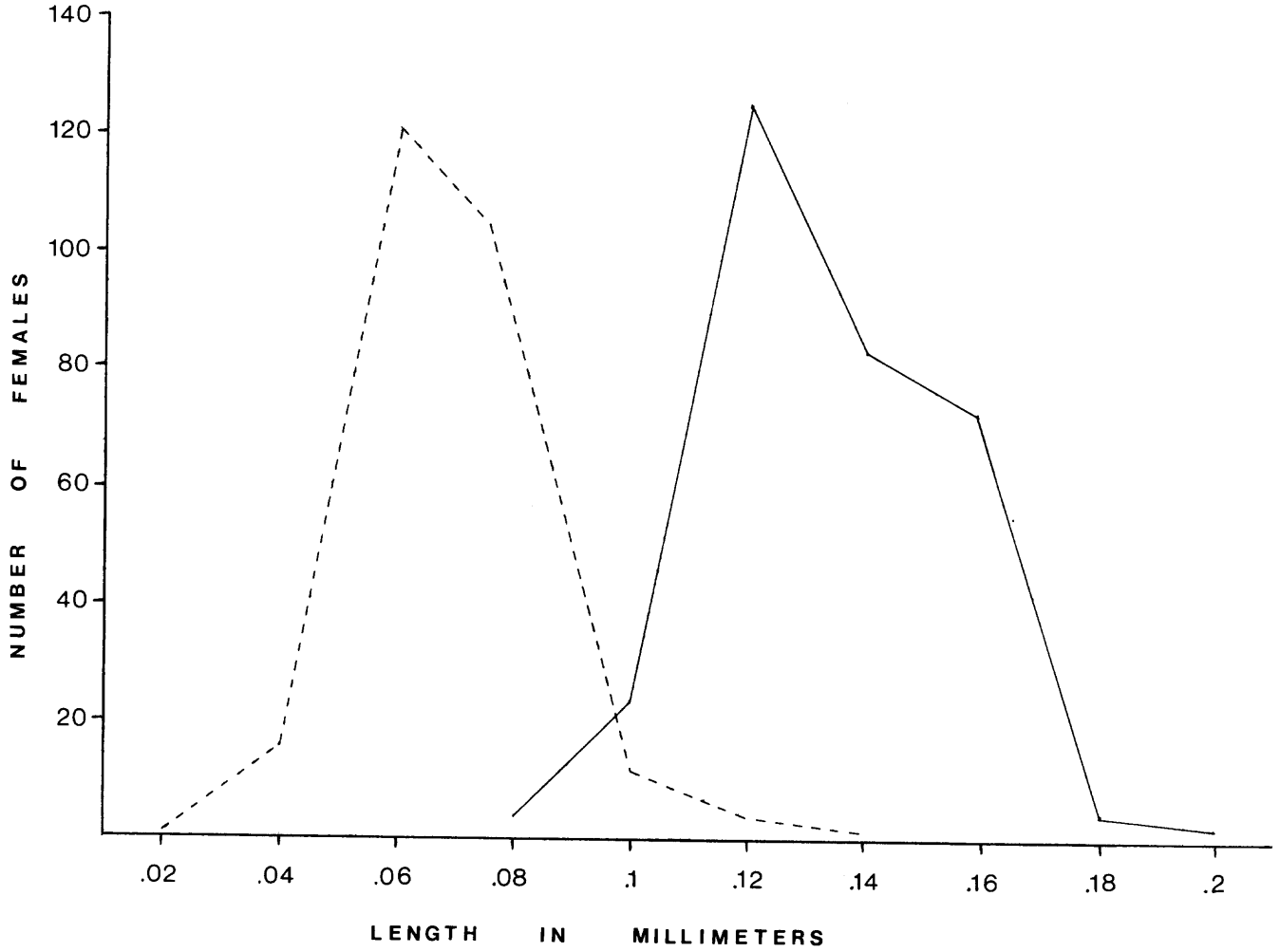

Figure 1. Distribution of the leg banding measurements of *Anopheles gambiae/arabiensis* (dotted line) and *merus/quadriannulatus* (solid line).